EMERGING INFECTIONS: LESSONS FROM THE VIRAL HEMORRHAGIC FEVERS

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ABSTRACT

Two Institute of Medicine reports since 1992 have emphasized the dangerous and continuing threat to the world from emerging infectious diseases. Working with viral hemorrhagic fevers provides a number of lessons related to the processes that control emergence, the pattern of disease after emergence, and how to cope with these incidents. This short paper uses two arenavirus hemorrhagic fevers to illustrate some of these principles. Argentine and Bolivian hemorrhagic fevers first came to medical attention in the 1950's. The forces that underlie the emergence of disease in Argentina are not understood, but the Bolivian episode has a reasonably understandable train of events behind it. The Argentine disease had serious impact on the large agricultural economy, and the ecology of the rodent reservoir did not lend itself to control; a vaccine was developed by Argentina and the U.S. with the latter motivated largely by biodefense. The Bolivian disease was controlled in large part by eliminating rodents that invaded towns, and the impact was subsequently below the level needed to trigger drug or vaccine development. These two viruses were important in the recognition of a new family of viruses (*Arenaviridae*), and this finding of new taxons during the investigation of emerging infectious diseases continues.

Introduction

In 1992, the Institute of Medicine published a report referring to Emerging Microbial Threats. That report (1) was the wake-up call for the general medical and research community to see that there are rapidly changing patterns in infectious diseases and that there are identifiable forces behind this shifting landscape. A decade later a follow-up report was issued (2) and this report reaffirmed the major factors involved and warned that the different factors underlying the threat were moving at an increasing pace. The major addition to their report was an emphasis on the interacting nature of the forces so that the resultant risk was multiplied. The original reports give more

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detail, but I distill the risk factors into a simple equation (Table 1). In the case of most viral infections, I would argue that the driving forces are the availability of multiple new ecologic niches plus the facility with which viruses and their reservoirs are moved around the globe by travel and transport (3). Mutations in the viral genome that may occur are usually secondary to adaptation to the new niche. I will illustrate this thesis with some examples drawn from the epidemiology of the viral hemorrhagic fever (VHF) syndrome. Counter examples are viral adaptation of the severe adult respiratory syndrome virus (4) and the driving role of viral evolution in emergence of human epidemics of influenza A virus (Discussed by Dr. John Bartlett in this volume).

VHF comprise a group of similar clinical entities caused by viruses from 4 different families (Table 2) (5). These diseases differ in their pathogenesis, and their causative viruses differ in their replication, maintenance, epidemiology, and host interactions. All are maintained in nature, and human infections are of little consequence to the virus' strategy for survival. In nature, they cause chronic infections in at least one of their hosts, vertebrate, or arthropod; but in humans, they cause acute infections without chronicity and usually without recognized sequelae.

The arenaviruses are a family of viruses that are chronic infections of rodents in Africa and the Americas. The prototype virus for the family, *Arenaviridae* is lymphocytic choriomeningitis virus, which has contributed so much to our understanding of immunology. However, the viruses we are going to discuss are two South American viruses, Junin and Machupo. Their stories show some of the features of emerging infections and of rodent-borne diseases.

THE REMARKABLE APPEARANCE OF ARGENTINE HEMORRHAGIC FEVER

Junin virus causes Argentine hemorrhagic fever (AHF), which was first recognized in the early 1950's by an Argentine clinician named Arribalzaga (6). He described a characteristic febrile syndrome with thrombocytopenia and hemorrhage in agricultural workers of the pam-

TABLE 1
Forces behind emergence

Genetically variable viruses + Multiple ecologic niches + Global travel and transport	=	Evolutionary opportunities for viruses

TABLE 2
Viral hemorrhagic fevers

Arenaviridae[a]	
	Lassa fever[b]
	South American HF (Argentine, Bolivian, etc)
Bunyaviridae	
Phlebovirus[c]	Rift Valley fever
Nairovirus	Crimean Congo HF
Hantavirus	HF with renal syndrome
	Hantavirus pulmonary syndrome
Filovirus	
	Marburg HF
	Ebola HF
Flavivirus	
	Yellow fever
	Dengue HF
	Kyasanur Forest disease and Omsk HF

[a] Virus family.
[b] Disease name.
[c] Virus genus.

pas near the town of Junin that was sufficiently characteristic that he believed it to be a new disease. The causative virus (named Junin virus) was soon isolated by Argentine virologists from academia and public health sectors (7, 8) and found to be a rodent borne virus which later became a charter member of the arenavirus family. The geographic extension of the disease and the number of cases increased progressively over the years (9). The clinical disease and its treatment by convalescent plasma were studied during this time, but morbidity and mortality was substantial. The reason for the initial emergence or the spread of the disease remained a matter of intense speculation, but none of the competing hypotheses was ever proven: crop type, ground cover, type of cultivation, use of defoliants, rodent density. By 1988, several hundred cases were occurring annually with no prospects for control.

A candidate vaccine had been developed by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) and the Argentine public health authorities. USAMRIID participated because of the threat of Junin and Machupo viruses as a biological warfare agent, and there was evidence that a vaccine against one would protect against both. The Argentine government was motivated by the impact of Argentine hemorrhagic fever on workers in the agriculture industry. The pampas, where Junin virus circulates, are extremely important to the nation's economy, and the disease had become a political issue. The two countries participated together in the

development of the vaccine and later undertook an extensive collaborative field trial which proved successful, providing >95% protection (10, 11). During the trial, it was possible to perform several studies of rodents and to make some pertinent observations on virus transmission:

1. During the first year of the trial rainfall was very low and there were almost no human cases (12). This was bad news for the trial, as accrual was too low to be useful, but it provided the best example of the correlation between arenavirus disease and rodent populations, which were greatly reduced.
2. The number of rodents/hectare was the same in the areas ahead of the progressive wave of expansion, in the recently involved "hot" areas, and in the "burned out" areas that had been involved historically (13). However, in the three different zones, rodent infection prevalence was vastly different and corresponded to human infection incidence. Ahead of the hot area, where there was no human disease, rodent infection was absent or very rare. In the hot area, rodent infection was 5–10% and humans were infected at a rate of 1–2/1000/yr. Behind the hot area, human disease and rodent infection were low. Thus, the spread of the virus accounted for the spread of the disease but was not associated with increases in rodent population densities nor was the decrease in the older endemic areas caused by a decrease in rodent populations.
3. The observation was made that the rodent reservoir of Junin virus, *Calomys musculinus*, was almost exclusively found along the fence lines, roadsides, and other linear habitats. This is the most extreme example of habitat segregation in a rodent-borne disease reported. It also provided a new risk management strategy for AHF.

The questions of why AHF arose as a clinical disease, and why its incidence and distribution enlarged, remain unanswered. We understand more about its ecology and how to avoid infection based on rodent habits. However, the vaccine has now been given to >250,000 persons at highest risk in the endemic area with a decrease in annual disease incidence below 100 cases.

BOLIVIAN HEMORRHAGIC FEVER (BHF) AND ITS CHANGING PATTERNS

In the late 1950's changing politics in Bolivia led to a systematic attempt by the government to settle the Beni Department for smallhold agriculture. The Beni is a flat zone near the Amazon River that

also features elevated areas where small towns may be built. During 1959–1962, isolated cases of a disease referred to as "black typhus" were noted principally among men in these remote areas. The disease pattern took a more sinister turn in 1962 when it invaded a town on an island in the Orobayaya River. This small town of 600 persons sustained 107 cases with 44 deaths (41% case-fatality). Investigations by the Ministry of Health and local physicians failed to reveal an etiology or develop a control strategy, and the inhabitants abandoned the town.

The next year cases appeared in San Joaquin, a town of about 3000. During 1963–64 there were 637 cases (21% of the inhabitants) and 113 of these died (18% case fatality). A team from the Middle America Research Unit (an NIAID laboratory in Panama) supported by Department of Defense went to the site to work on the disease cause. The causative agent was isolated and proved to be a previously unknown virus that was subsequently named Machupo virus for the nearby Machupo River (14). Extensive studies of mammals and arthropods yielded only isolates from rodents, particularly *Calomys callosus* and the inference was made that Machupo virus chronically infected rodents and was spread directly to humans (15). The definitive link was made by trapping rodents from half the town and finding the disappearance of the disease in that area approximately 2 weeks later with continuing disease in the untrapped zone (16). This maneuver made a sufficient impression that even today towns are kept free of *C. callosus* by trapping, usually using persons immune to BHF by virtue of recovering from the disease. Several years later Junin, Machupo, and several other viruses were grouped together into a new virus family, *Arenaviridae* (17).

The story of BHF is not over. The remarkable proclivity of *C. callosus* to enter towns was responsible for the town-based epidemics, but sporadic cases have continued to occur. Some of these cases are on the edge of towns suffering some rodent incursions. Others are in persons leaving town to work in their agricultural fields in clearings near the towns. Most interesting is the consistent finding of inter-human transmission (18–20). This pattern of spread was not suspected during the town epidemics, probably because the intensity of direct rodent-human transmission seemed to account for multiple cases within households.

Little is understood of the ecological determinants of human disease, and nothing beyond the trapping of town-based rodents is available for disease control. Ribavirin probably is effective in the disease in animals and in humans (21, 22), but is not available for general use in the endemic zone. There is animal evidence that the Junin vaccine protects against Machupo virus challenge, but no human studies have been

carried out and the vaccine is not in use in the endemic area (C. J. Peters and Peter B. Jahrling, unpublished observations).

SOME LESSONS

These are case studies of diseases caused by chronic rodent virus infections and do not require virus mutation to lead to human disease emergence. Perhaps they are not the best examples to probe the issues around virus genetic change. Nevertheless, they do demonstrate a number of common principles in emergence:

1. When new infectious diseases emerge, they are usually identified by the clinician, which puts a special responsibility on us all.
2. Once recognition occurs, epidemiologic and etiologic studies are needed to define the problem. Microbiology is usually the most direct and definitive route to the answer.
3. In the case of viruses, the agent is often a "new" (to science) virus. It may even represent a new family or a new genus previously undescribed. It will often have relatives with certain similarities.
4. Land use and human behavior are often important in emergence (1)
5. Ecological factors are important and include rodent behavior that obviously dictated the epidemiology of the emergences of AHF and BHF.
6. Identification of the problem requires ecological and other research that will hopefully give routes to ameliorate the disease.

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DISCUSSION

Runge, Chapel Hill: With the hemorrhagic fever viruses, what is it about the rodent immune system that seems to allow them to become endemic and not wipe out the rodent populations?

Peters, Galveston: No, the arenaviruses and hantaviruses are both chronic infections of rodents. The rodents actually do very well. With some of the arenaviruses there is a decreased fertility in chronic rodent infections, which may be one of the explanations we could try to apply to the moving front of Argentine hemorrhagic fever virus. We worked quite a bit with hantaviruses in the southwestern U.S. following 1993. In trap and release studies, their growth in the wild infected is the same as uninfected: normal weight gain, normal duration of life span, and we think normal fecundity, although our numbers aren't as good for that. So the way the rodent and the virus adjust mutually is unknown. These are all out-bred animals. They are very hard to work with because of the need to colonize them and develop all the markers used with laboratory mice. If you take lymphocytic choriomeningitis virus, the type member of the arenavirus family, as an example, we can say something about that type of virus-rodent relationship. Lymphocytic choriomeningitis virus (and apparently Lassa virus, as well) suppresses the host immune system selectively. They may become chronically viremic for life. On the other hand, the hantaviruses cause a transient viremic infection. They are cured in terms of the systemic effects of the virus, but it retains its ability to be excreted on mucosal surfaces, particularly in urine. Some sort of selective tolerance must be involved, and we don't understand it at all.

Cohen, Chapel Hill: You focused, in talking about these agents, on animal-to-human transmission, but, of course, there is a tremendous fear in this country of the use of these agents for bioterrorism. Could you comment on that?

Peters: Thanks for bringing that up. I left that out of my introduction. One of the unusual features of these viruses is that they are virtually all aerosol infectious. Different viruses differ greatly in their ability to infect by aerosol. For some reason we don't understand, hemorrhagic fever viruses are capable of causing serious aerosol infections. And that results in two corollaries. One corollary is that when you work with them, you need a laboratory setting that is highly protective against aerosols such as a biosafety level 4 laboratory—which combined with poor infrastructure in the countries where the hemorrhagic fevers occur, low funding, and hazard to the laboratory worker further reduces the amount of work that's done on these viruses. And the other corollary is that you can generate aerosol clouds and they make ferocious biological weapons. The U.S. was working on weaponizing Rift valley fever in 1967 when we, thankfully, disestablished our program. And the Soviets actually weaponized Marburg virus, and that was one of their particularly unpleasant weapons.

Johnson, Ponte Vedra: Thanks for your interesting talk. I know that you know

something about the filoviruses, but your slide had a question mark about the reservoir. Would you like to speculate about the reservoir for Ebola and Marburg and give us your thoughts about that?

Peters: That's the only known virus family where we just don't understand the natural history, and we have looked for the reservoir on a few occasions and a few of these studies have been exhaustive. One of the problems is that filoviruses occur in tropical areas and the epidemics are typically generated when a single person is infected. Then it's transmitted to a caregiver within a family, and finally it gets into a hospital. In an African hospital without sterilization of equipment you have a massive, explosive epidemic. By the time we get there, we may have heard about the epidemic from African sources, but we have to wait for someone to think to take a lab sample for diagnosis. By the time you get there, you follow a cold trail back to the index case, who is the link to the true reservoir. You investigate within an ecologically complex forest. We did 3,000 rodents in 1995 after the Kikwit, Zaire outbreak. And we didn't find any virus, but we did find three new species of shrews, which gives you an idea of how difficult it is to work in this environment where you can't really understand the complexity that you have to deal with before hand. There are various things that have led us to speculate that bats could be important. And probably the single best clue to that, to my way of thinking, is a Marburg outbreak that occurred in the towns of Durba and Watsa in former Zaire, now the Democratic Republic of the Congo. A friend of mine was dying to isolate a virus from that outbreak and call it "whatzavirus", but it, unfortunately, turned out to be Marburg. The disease appeared to be confined to a single activity: going into underground gold mines and working in these abandoned Belgium gold mines. And if the roof didn't fall in on you, there was a certain chance that you would leave the mine with Marburg virus, and then you would have secondary transmission. That's the only time we have had the opportunity to see repeated primary infections. And we were able to implicate the cave, but because of a good deal of civil unrest, we were never able to take the proper number of samples from the bats that we suspected of being the reservoirs.

Douglas, Niantic: I was curious about person-to-person transmission, perhaps by infected urine or respiratory secretions. And one of the reasons for this was that in the early 60's, when I was a clinical associate at the NIH, I took care of Pat Webb, who had visited Karl Johnson when he acquired Bolivian hemorrhagic fever working in the fields in Bolivia, and was being cared for at Gorgas Army Hospital in Panama. She visited him there and acquired the disease from him in a setting where human-to-human transmission in some fashion, is at least what we believe occurred. And you know, with regard to bioterrorism if an epidemic can propagate, it can be much more serious than if everybody else has to be infected by primary inoculation of the infected aerosol.

Peters: One of the things that was interesting about Pat Webb's case, is that it was the first example of inter-human transmission. Karl Johnson and others on the team working in Bolivia had always felt that Bolivian hemorrhagic fever was not transmitted person-to-person. When they finally cleaned the reservoir rodents out the town, and someone was infected in a corn field or yucca plantation, and came into town and got sick, then we could very clearly pin down person-to-person transmission. But it's not terribly common in Bolivian hemorrhagic fever. If you look at the hemorrhagic fever viruses that have been transmitted person-to-person, you find that the South American hemorrhagic fevers are only occasionally a risk, and there may be blood contamination or probably aerosol as well. Ebola and Marburg are really only occasionally spread in a modern hospital setting, but in an African hospital, it's a serious problem. Filoviruses are probably not transmitted person-to-person by aerosols, but rather by droplets or fomites. This is not to say that if small-particle aerosols are generated in the laboratory or intentionally as a bioterrorist effort, that they will not be an efficient mediator of infection.